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## Nest Temperatures and Hatchling Sex Ratios from Loggerhead Turtle Nests Incubated Under Natural Field Conditions in Georgia, United States

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**ABSTRACT.** – We examined loggerhead nest temperatures and hatchling sex ratios in an effort to more accurately predict hatchling sex ratios produced from 2 barrier islands in the northern management unit (Blackbeard Island National Wildlife Refuge and Wassaw National Wildlife Refuge, Georgia, United States) from 2000 to 2004. Temperature data loggers were placed into 169 nests to monitor incubation temperatures. Average critical period temperatures ranged from 26.3°C to 33.2°C (mean  $\pm$  SE, 29.2°  $\pm$  0.1°C) and indicated seasonal variation in sex ratios. The sex of 669 hatchlings found dead in nests was histologically evaluated ( $n = 212$  nests; 14–90 nests/yr). The sex ratios varied from 0% to 100% female per nest ( $n = 1$ –53 hatchlings/nest) and average sex ratio for all nests ranged from 55.5% female in 2003 to 85.4% female in 2002. In addition to monitoring nest temperature, 10 hatchlings per nest were euthanized to verify sex during 2003 on Blackbeard Island National Wildlife Refuge ( $n = 10$  nests) and 2004 on Wassaw National Wildlife Refuge ( $n = 9$  nests). Sex ratios were analyzed by using an advanced statistical program for evaluating temperature-dependent sex determination and indicated a 1:1 temperature (temperature that produces a 1:1 sex ratio) of 28.9°C. We offer an equation for predicting northern management unit hatchling loggerhead sex ratios by using critical period temperature and tested its validity. Sixteen of 18 nests ( $n = 10$  hatchlings/nest) showed no significant difference between the predicted sex ratios based on the equation vs. sex ratios obtained through histology. Our data indicated that rookery beaches north of Florida are important areas for the production and recruitments of male loggerhead hatchlings into the overall western North Atlantic Ocean and nests deposited earliest within a nesting season are primary contributors of male turtles. We suggest that nest monitoring programs grant such nests particular protection to increase their survivability and the production of hatchlings.

**KEY WORDS.** – Reptilia; Testudines; Cheloniidae; *Caretta caretta*; loggerhead; hatchling sex ratio; temperature-dependent sex determination

Loggerhead sea turtles, *Caretta caretta*, are listed as a threatened species under the US Endangered Species Act. Genetic analyses of nesting turtles in the southeastern United States indicate that there are 5 subpopulations and/or management units within this region (Bowen et al. 1993; Encalada et al. 1998; NMFS and USFWS 2007). These subpopulations appear to be declining despite federal protection efforts initiated for this species in 1979 (NMFS and USFWS 2007). Consequently, it has become imperative for managers and conservation groups to elucidate all aspects of the life history and ecology of loggerhead turtles within each specific subpopulation (Plotkin and Spotila 2002). Such data are necessary for developing accurate recovery plans that address the particular life history attributes and threats associated with each regional loggerhead turtle management unit (NMFS and USFWS 2008).

Ascertaining the factors associated with nest success and hatchling production is of particular importance for

managers to assess and predict the affects of anthropogenic activities on individual loggerhead rookery beaches (NMFS and USFWS 2008). Many attempts to do so, however, use models derived from laboratory studies and may not accurately reflect the results generated by nests incubated in the natural environment (i.e., Mrosovsky 1988; Mrosovsky and Provancha 1989). The present study examines whether the results of such studies are valid predictors for sex ratios of nests incubated in the field.

Loggerheads exhibit temperature-dependent sex determination (TSD) (Yntema and Mrosovsky 1980). Current information indicates that loggerheads appear to fit in an overall trend of female-biased sex allocation, which occurs in many reptiles with TSD (reviewed in Freedberg et al. 2001). There are 3 patterns of TSD, and sea turtles are categorized as exhibiting a pattern known as “pattern Ia” (Pieau et al. 1995). Animals that exhibit TSD pattern Ia yield a 1:1 sex ratio when incubated at a pivotal temperature. Eggs incubated below the pivotal temperature

will produce mostly males, and temperatures above the pivotal will produce mostly females (Bull 1980; Mrosovsky and Yntema 1980; Mrosovsky and Pieau 1991). Previous laboratory studies have defined the term “pivotal temperature” to indicate the constant incubation temperature that produces a 1:1 sex ratio. In the current study, nest incubation temperatures experienced natural fluctuations because the nests were located on the beach. Therefore, the term 1:1 temperature will be used to indicate the average of the daily fluctuating temperatures that produces a 1:1 sex ratio. Our study reports the 1:1 temperature and temperature-associated sex ratios derived from nests incubated on rookery beaches in Georgia, United States. These data are compared with previous studies that use different methods to examine the loggerhead nest environment. We also demonstrate the need to supplement existing methods with those introduced in this study to better ascertain the sex ratios produced in loggerhead nests. The management implications of our data are also presented and discussed.

## METHODS

*Study Sites.* — This study was conducted on Wassaw National Wildlife Refuge (WI) and the Blackbeard Island National Wildlife Refuge (BBI), each located off the coast of Georgia. WI is located 12 km south of the South Carolina–Georgia border, off the coast of Richmond Hill, Georgia (lat 31°87'N, long 80°97'W); BBI is approximately 90 miles south of Savannah, Georgia, off the coast of Valona, Georgia (lat 31°30'N, long 81°12'W). These 2 barrier islands are national wildlife refuges, maintained by US Fish and Wildlife Service. WI and BBI have 6.6 and 8.9 miles of beach, respectively. These islands experience high daily tidal fluctuations (sometimes > 2.4 m) (Johnson et al. 1974). BBI is known to have the highest nesting density of loggerhead sea turtles on the Georgia coast and has had established nest protection programs since 1965 (Dodd and Mackinnon 2003).

*Field Study.* — Female *C. caretta* nest on the Georgia coast from mid May until early August. Nests hatch from late June until early October. WI was monitored each year from 2000 to 2004, and BBI was monitored from 2001 to 2003. The majority of nests were deposited in mid June and hatched in mid August. This study was conducted over 5 seasons (2000–2004) to ensure that the collection of temperature and sex ratio data was not from an atypical year and was evenly distributed throughout the entire range produced on these natural beaches (as mentioned in Mrosovsky and Provancha 1989, 1992; Mrosovsky 1994). We monitored BBI and WI with nightly beach patrols throughout the entire loggerhead nesting seasons for the years monitored during this study.

Nests that had a high probability (> 80%) of seawater inundation due to high tidal fluctuations were relocated to higher parts of the beach to prevent total loss (US Fish and Wildlife Service protocol). As a nest

predator deterrent, all the nests were covered with wire screening for the duration of their incubation.

*Temperature Data Loggers.* — Nest temperatures were monitored both on WI (2000–2004) and BBI (2001–2003). Calibrated 2K HOBO® temperature data loggers (Onset Computer Corp) were placed in each of 169 nests (77 in situ, 92 relocated nests) to record a temperature profile throughout the entire incubation period. Data loggers were placed on their side within the center of the egg mass to record temperature (°C) once every 2 hrs. Temperature data were downloaded by using Box Car® 3.6 Software (Onset Computer Corp) and then were exported into JMP statistical software version 4.0.4 (©SAS, 2001). During the 2001 nesting season, a destructive storm washed away many nests with data loggers, which reduced the total number for that year. Analyses that involved temperature did not include WI 2004's nest no. 27 because the data logger did not record the temperature data properly. Relocated and in situ nest temperatures were not found to be significantly different by Tuttle and Rostal (2010) and thus were combined for analyses.

*Determining Sex of Hatchlings.* — Nests were excavated after hatching. Any dead hatchlings found in a nest were collected and dissected so that the gonadal region was exposed and then were stored in 10% histology grade formalin. Dead hatchlings were collected on WI (2000, 2002–2004) and BBI (2001–2003). In 2000, dead hatchlings were obtained from 26 different nests deposited on WI between 14 May and 18 July. In 2001, dead hatchlings were obtained from 14 different nests on BBI deposited from 17 May to 23 June. In 2002, dead hatchlings were obtained from 63 different nests on BBI deposited between 7 May and 20 July, and from 5 different nests on WI deposited from 12 May to 28 June. In 2003, dead hatchlings were obtained from 84 different nests on BBI deposited between 7 May and 29 July, and from 6 different nests on WI deposited from 7 June to 2 July. In 2004, dead hatchlings were obtained from 14 different nests on WI deposited between 26 May and 25 July.

In addition to dead hatchlings, 10 live hatchlings were euthanized from 19 relocated nests that contained data loggers ( $n = 10$  nests, BBI 2003;  $n = 9$  nests, WI 2004). Nests were surrounded with a wire cage, and once all the hatchlings emerged, 10 hatchlings were randomly selected. Hatchlings were selected and euthanized from nests deposited between 30 May and 11 July. Although euthanizing a threatened species is never the first choice, these hatchlings were euthanized in the hope that this study would determine if dead-in-nest hatchlings represent an accurate sex ratio estimate.

Laboratory histology was performed to determine the sex of the dead-in-nest and euthanized hatchlings (Wibbels et al. 1999; based on Yntema and Mrosovsky 1980). Tissues were stained by using the histologic dyes hematoxylin and eosin (Humason 1972). The gonads

(both ovary and testis) show sex-specific characteristics (Yntema and Mrosovsky 1980; Merchant-Larios 1999). These data were compared with sex ratio predictions, based on nest temperatures.

**Data Analysis.** — Incubation duration was measured, in days, as the length of time from egg deposition to hatchling emergence. Mean temperatures ( $\pm$  SE) were calculated for each day during the entire incubation period for each nest. The mean temperature from the entire length of the incubation period is referred to as mean incubation period temperature (IPT). The middle third of the entire incubation period was approximated to be equal to the critical period, or thermosensitive period, for the loggerhead turtle (Yntema and Mrosovsky 1982) and is referred to as mean critical period temperature (CPT). Mean IPT and mean CPT were examined for homogeneity of variances before statistical analysis. If the homogeneity of variances assumption was not met, then a  $\chi^2$  test was run instead of analysis of variance (ANOVA). Mean IPT and CPT values were compared with a paired *t*-test.

Annual sex ratios, Julian date of oviposition, incubation duration, and mean temperature (IPT and CPT) were obtained by taking the sum of all females divided by the total number of hatchlings for which sex was determined during that period (i.e., percentages were not averaged). In addition, sex ratios per year were also calculated by taking the mean of all sex ratios given per nest (i.e., mean of average sex ratios per nest). All hatchlings that were collected dead from nests, not just from nests that contained a data logger, were used for calculating the overall sex ratio for each year.

A total of 190 hatchlings were euthanized: 100 from BBI (2003) and 90 from WI (2004). Sex ratio analyses used the percentage of female hatchlings from euthanized nests. Analyses also were run, which excluded nests that encountered abnormal rainfall and data logger malfunction. The numbers of males and females were sorted by the mean CPT and the incubation duration separately. The percentage of female per year was calculated by taking the mean of all sex ratios per nest (i.e., mean of individual nest sex ratios).

Both data sets, dead-in-nest and euthanized, were evaluated by using the TSD program developed by M.H. Godfrey, V. Delmas, and M. Girondot, and based on statistical methods outlined in Girondot (1999) and available from the URL: <http://www.ese.u-psud.fr/epc/conservation/TSD/index.html>. This program assumes that the sex ratios are distributed in a male-female pattern of TSD (which includes temperatures that produce the following: 1) an all-male range, 2) a transitional range of temperatures (TRT) in which sex ratios change from all-male to all-female and includes a pivotal temperature, which would produce a 1:1 sex ratio, and 3) an all-female range) and fits the data to the equation that best described its distribution by using maximum likelihood. This program also assumes that the curve that describes the

male-female pattern of TSD is symmetric around the pivotal temperature. This program evaluates the best fit model by generating an Akaike information criterion (AIC) value (Girondot 1999; Burnham and Anderson 2002). The lowest AIC value has the best fit to the data and thus provides the most accurate description of the data, i.e., the pivotal temperature and transitional range of temperatures. Equations whose AIC values are within 2.0 units of each other are not significantly different (Girondot 1999). The program estimates the pivotal temperature (P), the shape of the transition from masculinizing to feminizing temperature (S), and an asymmetrical shape parameter (K). For each parameter reported, including the AIC value, the average and standard deviation of 10 iterations are given. The power of the test ( $K = 0$  vs.  $K \neq 0$ ) was measured by using the program's parameters, with 100 replicates as suggested by the TSD program; a *p* value less than 0.05 indicates that the sample size is too small to differentiate between a symmetric and asymmetric shape of the curve between sex ratio and temperature. The alpha risk, or type I error, was also measured for the fit of the equation by using the parameters of the program, with 1000 replicates as suggested by the TSD program; a *p* value greater than 0.05 indicates that the fit cannot be rejected. The sums of male and female hatchlings per temperature were used for analyses. Both data sets were assigned an uncertainty of 0.4°C to account for the temperature precision of the data loggers.

The euthanized data set did not contain any low temperatures that would produce all males. Temperatures of 26°C or below are reported to produce all males in every sea turtle species examined to date (Wibbels 2003); in addition, temperatures as high as 28°C are shown to produce all males in some species (Wibbels 2003). Therefore, to anchor the curve on the low temperature end, a 100% male datapoint was added at 26°C to facilitate the program's calculation of the curve.

The Hill equation and parameters suggested by the TSD program (Girondot 1999) for predicting loggerhead sex ratios (sr), at a given temperature (CPT) from the dead-in-nest data set is as follows:

$$sr(t) = \frac{1}{1 + e^{(1/S)(\ln(P+K) - \ln(t+K))}}$$

$$S = -0.02571$$

$$P = 28.85814$$

$$K = 0.355112$$

Sex ratios were predicted based on critical period nest temperatures for euthanized hatchlings by using the formula provided by the Hill equation (TSD program) for the dead-in-nest data set. These predicted sex ratios were compared with the histologically obtained sex ratios by using the Fisher's exact test for each nest as well as the overall total of males and females.



**Table 1.** Mean nest temperature ( $^{\circ}\text{C}$ ) data from Wassaw National Wildlife Refuge (NWR) (WI) (2000–2004) and Blackbeard Island NWR (BBI) (2001–2003). HOBO temperature data loggers were placed in the center of the egg mass. Values are reported as means  $\pm$  standard error (SE) for the entire incubation period temperature as well as for the critical period temperature. No nests were monitored on BBI in 2000 and 2004. Comparison statistics are shown when both islands were monitored in the same year.

Yr	Location	No. nests	Incubation duration (mean $\pm$ SE)	Incubation period temperature, $^{\circ}\text{C}$ (mean $\pm$ SE)	Island comparison of incubation period temperature	Critical period temperature, $^{\circ}\text{C}$ (mean $\pm$ SE)	Island comparison of critical period temperature
2000	WI	17	52.6 $\pm$ 1.3	29.79 $\pm$ 0.17	—	29.72 $\pm$ 0.24	—
2001	WI	8	57.6 $\pm$ 1.2	28.36 $\pm$ 0.45	df = 25, $t$ = 2.313	28.45 $\pm$ 0.50	df = 25, $t$ = 1.898
2001	BBI	19	59.0 $\pm$ 0.9	29.11 $\pm$ 0.10	$p$ = 0.029	29.23 $\pm$ 0.17	$p$ = 0.069
2002	WI	24	57.5 $\pm$ 1.2	29.45 $\pm$ 0.29	df = 41, $t$ = 0.806	29.38 $\pm$ 0.35	df = 41, $t$ = 0.623
2002	BBI	19	55.9 $\pm$ 1.0	29.74 $\pm$ 0.16	$p$ = 0.425	29.66 $\pm$ 0.24	$p$ = 0.537
2003	WI	12	59.3 $\pm$ 0.8	28.94 $\pm$ 0.08	df = 49, $t$ = 1.643	28.61 $\pm$ 0.14	df = 49, $t$ = 1.940
2003	BBI	39	59.3 $\pm$ 0.6	29.24 $\pm$ 0.10	$p$ = 0.107	29.04 $\pm$ 0.11	$p$ = 0.058
2004	WI	31	59.2 $\pm$ 0.7	29.21 $\pm$ 0.11	—	29.25 $\pm$ 0.15	—

## RESULTS

**Temperature.** — The date of nest deposition influenced the IPT and CPT (IPT: linear regression, df = 1, 167;  $R^2$  = 0.096;  $p$  < 0.0001; CPT: linear regression, df = 1, 167;  $R^2$  = 0.078;  $p$  = 0.0002). No significant temperature difference between the 2 islands was found throughout the nesting seasons for 2001–2003 when both islands were monitored (Table 1). IPT differed between WI (28.36  $\pm$  0.27 $^{\circ}\text{C}$  [SE];  $n$  = 8) and BBI (29.11  $\pm$  0.18 $^{\circ}\text{C}$  [SE];  $n$  = 19) in 2001 ( $t$ -test: df = 25;  $t$  = 2.313;  $p$  = 0.029). However, IPT did not significantly differ between islands in 2002 or 2003, or for CPT for all 3 yrs (2001–2003) (Table 1).

Temperature did not significantly differ between the mean incubation period and the mean critical period during the 2000–2004 nesting seasons when comparing all nests (paired  $t$ -test:  $t$  = -1.600; df = 168;  $p$  = 0.112). For all nests monitored with a data logger during the 2000–2004 nesting seasons ( $n$  = 169), the mean ( $\pm$  SE) IPT was 29.3 $^{\circ}$   $\pm$  0.1 $^{\circ}\text{C}$  and the mean CPT was 29.2 $^{\circ}\text{C}$   $\pm$  0.1 $^{\circ}\text{C}$ .

There was a significant difference between each year's IPT (ANOVA:  $F_{4,164}$  = 4.91;  $p$  = 0.0009) and CPT (ANOVA:  $F_{4,164}$  = 3.06;  $p$  = 0.0182) (Table 2). Analyses revealed that IPT differed among years, when holding the Julian date as a covariate (analysis of covariance:  $F_{4,163}$  = 4.57;  $p$  = 0.002). An analysis of covariance could not be run to test for differences between the years in mean critical period temperatures, holding the Julian date as a covariate due to the lack of homogeneity of slopes.

Incubation duration ranged from 45 d (Julian date of oviposition, 199) to 71 d (Julian date of oviposition, 127), (mean, 57.88  $\pm$  0.36 d,  $n$  = 169) (Table 2). Incubation duration significantly differed between years (ANOVA:  $F_{4,164}$  = 9.349;  $p$  < 0.0001) but not between islands (BBI: mean, 58.4  $\pm$  0.5,  $n$  = 77; and WI: mean, 57.4  $\pm$  0.6,  $n$  = 92) ( $t$ -test: df = 167;  $t$  = 1.303;  $p$  = 0.194).

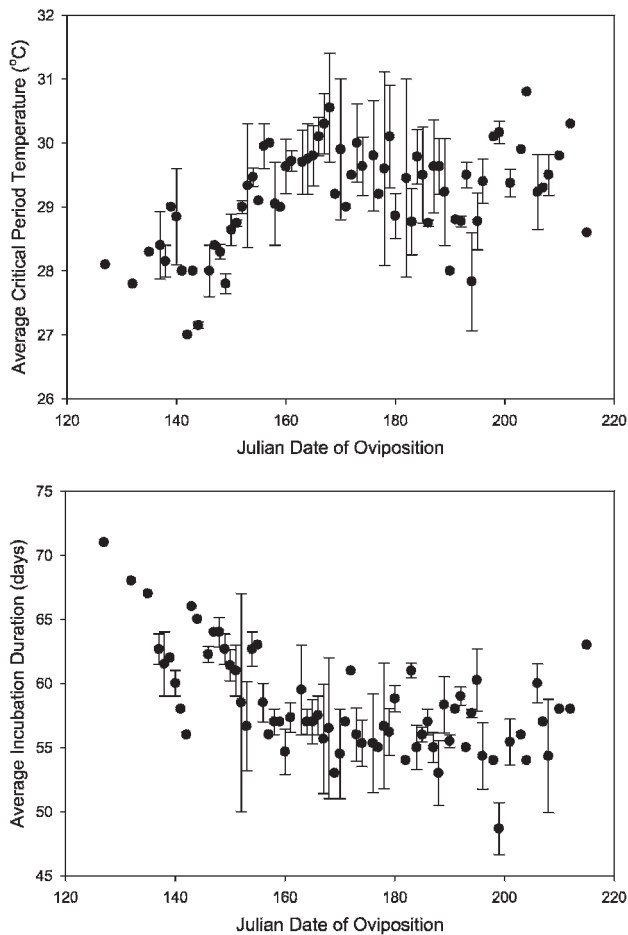
By using data from all years combined, incubation duration significantly decreased over the course of the nesting season (nonparametric Spearman signed rank test:  $n$  = 169;  $R_s$  = -0.417;  $p$  < 0.0001) (Fig. 1). In fact, there was a significant decrease for approximately the first 37 d (Julian dates, 127–164) (linear regression: df = 1, 60;  $R^2$  = 0.337;  $p$  < 0.0001) and then a leveling during the remaining 50 d (Julian dates, 165–215) (linear regression: df = 1, 105;  $R^2$  = 0.000;  $p$  = 0.868). This decrease in incubation duration corresponded with a significant increase in CPT during those first 37 d (Julian dates, 127–164) (linear regression: df = 1, 60;  $R^2$  = 0.395;  $p$  < 0.0001) and then a leveling during the remaining 50 d (Julian dates, 165–215) (linear regression: df = 1, 105;  $R^2$  = 0.026;  $p$  = 0.097) (Fig. 1).

Incubation duration decreased significantly with increasing mean IPT and CPT (IPT: nonparametric Spearman signed rank test:  $n$  = 169;  $R_s$  = -0.702;  $p$  < 0.0001; CPT: linear regression: df = 1, 167;  $R^2$  = 0.039;  $p$  < 0.0001) (Fig. 2).

**Sex Ratios.** — Sex ratios of hatchlings found dead in the nests after they had hatched were wide-spread throughout the nesting seasons. The average sex ratio per

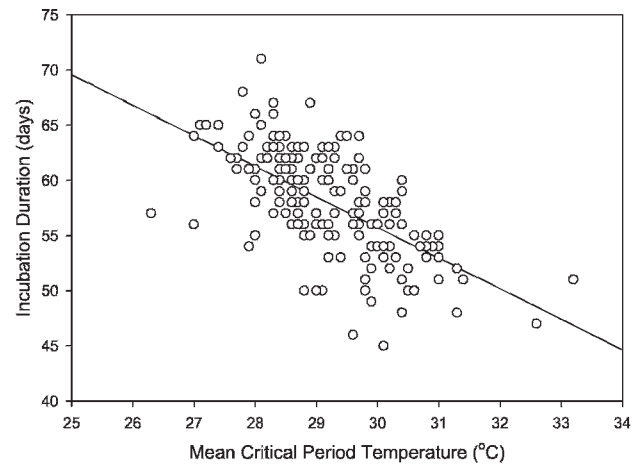
**Table 2.** Mean nest temperature ( $^{\circ}\text{C}$ ) and incubation duration (days) data from 2000–2004, separated by year. In 2000 and 2004, WI was the only location sampled. From 2001–2003, WI and BBI were sampled. HOBO temperature data loggers were placed in the center of the egg mass. Values are reported as means  $\pm$  SE (see Table 1 for definition of abbreviations).

Yr	No. nests	Incubation duration (mean $\pm$ SE)	Entire period (mean $\pm$ SE)	Critical period (mean $\pm$ SE)
2000	17	52.6 $\pm$ 1.3	29.78 $\pm$ 0.17	29.72 $\pm$ 0.24
2001	27	58.6 $\pm$ 0.7	28.89 $\pm$ 0.16	29.00 $\pm$ 0.20
2002	43	56.8 $\pm$ 0.8	29.58 $\pm$ 0.18	29.50 $\pm$ 0.22
2003	51	59.3 $\pm$ 0.5	29.17 $\pm$ 0.08	28.94 $\pm$ 0.10
2004	32	59.2 $\pm$ 0.7	29.21 $\pm$ 0.11	29.25 $\pm$ 0.15



**Figure 1.** Top: Critical period temperature (mean  $\pm$  SE) vs. Julian date of oviposition for all nests that contained a data logger from 2000 to 2004 on Wassaw and Blackbeard Island National Wildlife Refuges. Bottom: Incubation duration (mean  $\pm$  SE) vs. Julian date of oviposition for all nests that contained a data logger from 2000 to 2004 on Wassaw and Blackbeard Island National Wildlife Refuges.

nest was 67.4% female (average,  $3.1 \pm 5.6$  hatchlings per nest, for a total of 669 hatchlings). Twenty-six nests were sampled on WI in 2000, which resulted in the average sex ratio per nest of 60.3% female (average,  $2.3 \pm 1.9$  hatchlings per nest, for a total of 61 hatchlings). In 2001, 14 different nests on BBI were sampled and resulted in the average sex ratio per nest was 71.2% female (average,  $2.8 \pm 2.0$  hatchlings per nest, for a total of 39 hatchlings). In 2002, 63 different nests on BBI were sampled and resulted in the average sex ratio per nest was 85.3% female (average,  $2.5 \pm 2.8$  hatchlings per nest for a total of 166 hatchlings). In 2002, 5 different nests on WI were sampled (average  $3.6 \pm 2.7$  hatchlings per nest for a total of 18 hatchlings); the average sex ratio per nest was 86.7% female. In 2003, 84 different nests on BBI were sampled (average  $2.3 \pm 2.2$  hatchlings per nest for a total of 196 hatchlings); the average sex ratio per nest was 55.6% female. In 2003, 6 different nests on WI were sampled (average  $2.7 \pm 3.6$  hatchlings per nest for a total of 16 hatchlings); the average sex ratio per nest was



**Figure 2.** Incubation duration (days) vs. mean critical period temperature (linear equation:  $y = 138.83 - 2.77(x)$ ;  $n = 169$ ;  $R^2 = 0.392$ ;  $p < 0.0001$ ).

55.0% female. In 2004, 14 different nests on WI were sampled (average  $12.4 \pm 18.0$  hatchlings per nest for a total of 173 hatchlings); the average sex ratio per nest was 66.1% female (Table 3).

The percentage of females produced per egg deposition date did not follow a seasonal pattern for all years and for both islands combined (linear regression:  $df = 1, 69$ ;  $R^2 = 0.002$ ;  $p = 0.697$ ). These dead-in-nest hatchlings did show a correlation between the percentage of females produced and the incubation duration (linear regression:  $df = 1, 22$ ;  $R^2 = 0.669$ ;  $p < 0.0001$ ).

A total of 190 hatchlings were euthanized as part of this study and produced a sex ratio of 54.7% female. The sex ratio of the euthanized hatchlings was 49.0% female ( $n = 100$ ) in 2003 and 61.1% female ( $n = 90$ ) in 2004 on WI. The percentage of females produced per egg deposition date did not follow a seasonal pattern (linear regression:  $df = 1, 17$ ;  $R^2 = 0.063$ ;  $p = 0.301$ ). The euthanized hatchlings did not show a correlation between the percentage of females produced and the incubation duration (linear regression:  $df = 1, 17$ ;  $R^2 = 0.065$ ;  $p = 0.293$ ).

No significant relationship was found between the percentage of female hatchlings and IPT (linear regression:  $df = 1, 16$ ;  $R^2 = 0.077$ ;  $p = 0.264$ ) or CPT (linear regression:  $df = 1, 16$ ;  $R^2 = 0.203$ ;  $p = 0.061$ ). Two nests deposited on BBI in 2003 (nos. 113 and 164) experienced a heavy rainfall during or immediately before the middle third of their incubation period, which skewed the CPT data more than any other nests examined for this period. When these 2 nests were excluded from the analysis, the sex ratio of the remaining nests was significantly correlated with the CPT (linear regression:  $df = 1, 12$ ;  $R^2 = 0.390$ ;  $p = 0.017$ ) (Fig. 3). However, the sex ratio of the remaining nests was not significantly correlated with IPT (linear regression:  $df = 1, 14$ ;  $R^2 = 0.085$ ;  $p = 0.273$ ) or incubation duration (linear regression:  $df = 1, 15$ ;  $R^2 = 0.089$ ;  $p = 0.245$ ).

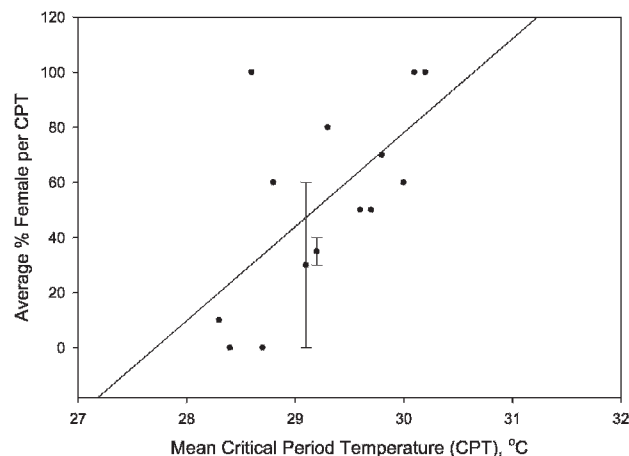
**Table 3.** Sex ratios of hatchlings found dead in nests (DIN) after they had hatched were widespread throughout the nesting seasons, given in Julian date (JD). In 2000 and 2004, WI was the only location sampled. From 2002 to 2003, WI and BBI were sampled. No hatchlings were collected on WI in 2001 (see Table 1 for definition of abbreviations).

Yr	Island	JD range	No. nests	Average no. DIN/nest	Total no. DIN	Average sex ratio/nest (% female)
2000	WI	135–200	26	$2.3 \pm 1.9$	61	60.3
2001	BBI	137–174	14	$2.8 \pm 2.0$	39	71.2
2002	BBI	127–201	63	$2.5 \pm 2.8$	166	85.3
2002	WI	132–179	5	$3.6 \pm 2.7$	18	86.7
2003	BBI	127–210	84	$2.3 \pm 2.2$	196	55.6
2003	WI	158–183	6	$2.7 \pm 3.6$	16	55.0
2004	WI	147–207	14	$12.4 \pm 18.0$	173	66.1
Overall		127–210	212	$3.1 \pm 5.6$	669	$67.4 \pm 40.2$

Data from the dead-in-nest hatchlings included 298 hatchlings from 149 nests and 35 average incubation temperatures. Temperatures from the data set ranged from 27.4°C to 33.2°C. By using data from the dead-in-nest hatchlings in the TSD modeling program, the estimated P, which we use as the 1:1 temperature, when using the Hill equation was  $28.9^\circ \pm 1.9^\circ \times 10^{-5}^\circ\text{C}$  ( $df = 33$ ;  $AIC = 70.50 \pm 2.1 \times 10^{-5}$ ). The TRT ranged from 26°–30.5°C (Fig. 4).

Data from the randomly euthanized hatchlings included 18 nests and 15 average incubation temperatures. Temperatures from the data set ranged from 28.3°–30.2°C. By using data from the randomly euthanized hatchlings in the TSD modeling program, the estimated P, which we use as the 1:1 temperature, when using the Richards equation, was  $28.9^\circ \pm 3.1^\circ \times 10^{-3}^\circ\text{C}$  ( $df = 13$ ;  $AIC = 62.28 \pm 6.2 \times 10^{-2}$ ). The TRT ranged from 27.5°C to 31.5°C (Fig. 4).

A summary of the data set analyses is shown in Table 4. The Richards and Hill distributions produced values that were similar and did not differ by more than 2 AIC units. For the dead-in-nest data set, the Hill equation generated the lowest AIC value relative to other models.



**Figure 3.** Percentage of female hatchlings vs. mean critical period temperature (°C) in nests from which hatchlings were euthanized, Blackbeard Island National Wildlife Refuge in 2003 (excluding nest nos. 113 and 164) and Wassaw National Wildlife Refuge in 2004 (linear equation:  $y = -966.65 + 34.77(x)$ ;  $n = 16$ ,  $R^2 = 0.351$ ;  $p = 0.0157$ ).

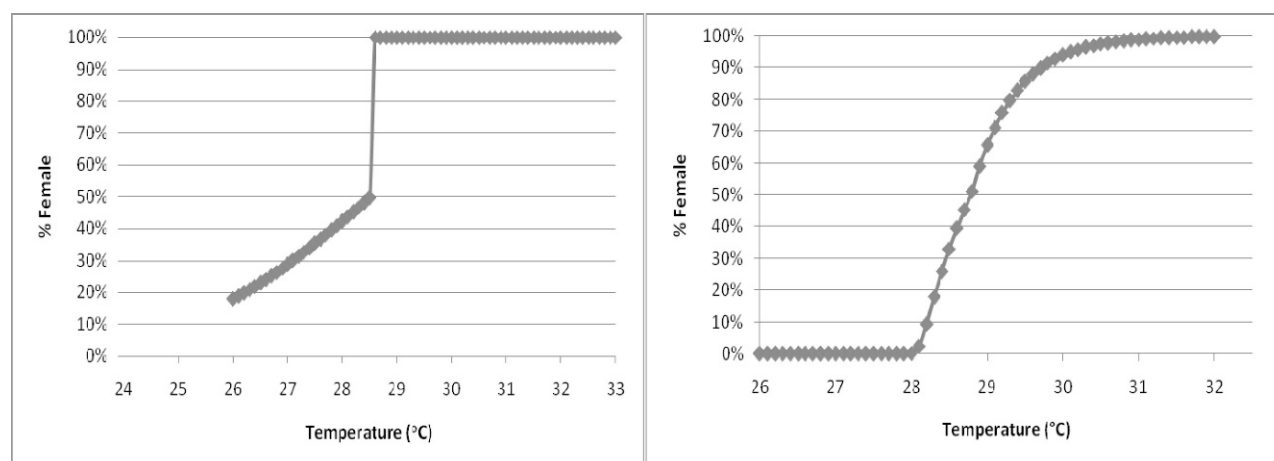
The Hill equation and parameters suggested by the TSD program (Girondot 1999) for predicting loggerhead sex ratios (sr), at a given temperature (CPT) from the dead-in-nest data set are provided in the Methods section.

Overall, predicted and histologically obtained sex ratios were female biased. The Hill equation and parameters suggested for hatchling loggerhead sex ratio predictions were used to predict sex ratios for the euthanized hatchling nest temperature data. Comparisons were made for each nest by using the Fisher's exact test. Sixteen of 18 euthanized nests ( $n = 10$  hatchlings/nest) showed no significant difference between the sex ratios predicted to be produced based on the above equation (18 nests combined predicted 105 females and 75 males) vs. sex ratios obtained through histology (18 nests combined produced 95 females and 85 males) (18 nests combined, Fisher's exact test:  $p = 0.340$ ).

## DISCUSSION

Seasonal variation was seen in critical mean temperature and incubation duration. This relationship has also been noted by Stokes et al. (2006). Male turtles appear to be produced primarily in the first 37 d and females for the remainder of the nesting season. These temperatures and incubation durations measured in this study both predict mixed sex ratios according to previous literature (reviewed in Wibbels 2003).

Bull (1980) stated that, for long-lived species, such as sea turtles, yearly extremes in hatchling sex ratios would have little effect on the population's overall sex ratio because yearly extremes would cancel each other. This study showed temperature differences among years (which produced variable sex ratios for each year), and the overall sex ratio from raw totals of dead hatchlings for all years remained female biased (61.6% female). The nesting seasons of 2000–2002 produced highly female-biased sex ratios, whereas 2003 produced a sex ratio closer to 1:1. Additional nesting season surveys are needed to determine whether the temperatures produced in 2003 were abnormal or if 2000–2002 were abnormally warm. Additional nesting seasons that experienced lower average temperatures would undoubtedly result in a lower overall female-biased sex ratio. In Georgia, 2000–2002



**Figure 4.** Left: Average sex ratio (% female) vs. temperature from Richards model determined by the temperature-dependent sex determination (TSD) program, based on 180 randomly euthanized hatchlings from 18 nests and 15 temperatures. Right: Average sex ratio (% female) vs. temperature from Hill model determined by the TSD program, based on 298 dead-in-nest (DIN) hatchlings from 149 nests and 35 temperatures.

were drought years, with lower than average rainfall amounts for the state and likely resulted in higher nest temperatures.

Many loggerhead populations around the world are also reported to exhibit predominantly female-biased sex ratios. Modeling of the Florida nesting subpopulation predicts an annual hatchling production of approximately 90% females (Mrosovsky 1994; TEWG 2000). Additional estimates and predictions from Florida indicate a hatchling production of female turtles above 90% female (Mrosovsky and Provancha 1989, 1992; Hanson et al. 1998). An analysis of the sex ratio of juvenile loggerheads from Florida indicated that 67.9% of the individuals were female (Wibbels et al. 1991).

Shoop et al. (1998) reported that 65.3% of stranded juvenile loggerheads and 62.7% of stranded adult loggerheads examined on Cumberland Island, Georgia, were females. Similarly, immature loggerheads along the entire East Coast of the United States were determined to exhibit female-biased sex ratios, based on testosterone levels (see Owens 1997). Estimates from Brazil and Cyprus are also female biased (Marcovaldi et al. 1997; Godley et al. 2001). Conversely, a male-biased sex ratio, of 24.1% female, was reported for juvenile loggerheads in Australia (Wibbels et al. 1987).

Previous studies that examined the sex ratios of hatchling sea turtles have been limited because of the protected status of sea turtles and the need to euthanize hatchlings to verify their sex (Merchant-Larios 1999;

Wibbels et al. 1999). Our results indicate that the sex of many dead-in-nest hatchlings can be determined by histology, depending on the state of their decomposition. The sexing of dead-in-nest hatchlings via histology could potentially preclude the need to euthanize hatchlings (Wibbels et al. 1999). However, the use of dead-in-nest hatchlings is dependent upon the hypothesis that the sex of a dead-in-nest hatchling represents a random sample.

Although our results support the use of dead-in-nest hatchlings for sex ratio studies, 2 limiting factors should be considered. First, the level of decomposition can limit the effectiveness of this method. The dead-in-nest hatchlings in the current study were collected 72 hrs after live hatchlings emerged from the nest, and this yielded usable histologic data in many, but not all, cases. A time period of 5 d between nest emergence and excavation is a typical regulation included in many sea turtle permits in Georgia and is intended to allow the nest to experience natural conditions throughout the incubation and emergence periods (M. Dodd, Georgia Department of Natural Resources *pers. comm.*, May 2002). However, such protocols lend to the increased decomposition of dead-in-nest hatchlings and the loss of potentially valuable data. Nests examined for the current study were excavated no later than 3 days after emergence. Earlier excavation of hatched nests could potentially decrease the problem of tissue decomposition in dead-in-nest hatchlings, but this could also generate logistical problems, such as what to do with weak hatchlings that are found during the

**Table 4.** Analysis summary of dead-in-nests (DIN) and euthanized data sets. Standard deviations for 1:1 temperature and Akaike information criterion (AIC) values were based on 10 iterations of each data set.

Data set	Model equation	Power	Alpha risk	df	1:1 Temperature $\pm$ SD, °C	AIC $\pm$ SD
DIN	Richards	0.13	0.037	32	$28.8 \pm 1.5 \times 10^{-3}$	$72.45 \pm 1.4 \times 10^{-3}$
DIN	Hill	0.03	0.001	33	$28.9 \pm 1.9 \times 10^{-5}$	$70.50 \pm 2.1 \times 10^{-5}$
Euthanized	Richards	0.02	< 0.001	13	$28.9 \pm 3.1 \times 10^{-3}$	$62.28 \pm 6.2 \times 10^{-2}$
Euthanized	Hill	0.07	0.001	14	$29.0 \pm 3.2 \times 10^{-6}$	$64.36 \pm 5.0 \times 10^{-5}$



excavation that would have ultimately died in the nest days later. Managers should consider whether such compromised hatchlings should be euthanized, not only for humane reasons, but also to retrieve data that are necessary to ascertain important trends in the overall population. Another option is captive rearing of any weak hatchlings.

A second limiting factor in using dead-in-nest hatchlings for sex ratio analyses is sample size. In nests with high emergence rates, the number of dead-in-nest hatchlings may be low, thus limiting the confidence of the predicted sex ratio. However, in the case of estimating a sex ratio for an entire nesting beach, it may be possible to partially compensate for this problem by examining large numbers of nests (Wibbels et al. 1999). Nevertheless, the current results provide a foundation for supporting the use of dead-in-nest hatchlings as a method for estimating beach sex ratios.

Sex ratio predictions were analyzed by using the most recent and advanced statistical model for evaluating temperature-dependent sex determination. We recommend using the Hill equation to estimate sex ratios of the dead-in-nest data set because this set encompasses 5 yrs of data, is the largest in terms of number of hatchlings, contains the greatest number of temperatures, and generates the lowest AIC value relative to other models. These 1:1 temperature estimates for loggerheads are similar to pivotal temperatures estimated for other sea turtles (approximately 29°–30°C, Wibbels 2003) and for western North Atlantic loggerheads as predicted by Mrosovsky (1988) (29°C), which supports the efficacy of the techniques used by the researchers in determining the TSD parameters. The dead-in-nest data suggest that incubation temperatures of approximately 26°C or lower will produce 100% males and temperatures of approximately 30.5°C or higher will produce 100% females. Temperatures falling between this range will produce mixed sex ratios.

The intention of the sex ratio prediction equation given is to help predict general sex ratios for a nesting beach. Because the pivotal temperature and TRT represent averages for the population (Dodd et al. 2006), sex ratios for individual nests may not precisely match what is predicted by temperature when using this model. However, the use of this model provides the most accurate estimates currently available.

This study used the dead-in-nest Hill equation to predict sex ratios based on critical period temperatures recorded in the euthanized hatchling nests. When compared with sex ratios that were histologically obtained, 88.9% were not found to be statistically different. As a result, we believe that this is a viable option for predicting sex ratios based on nest temperatures from the natural nesting beach. This method would allow for the evaluation and development of optimal management strategies relative to sex ratio production for this threatened species.

Because the largest US nesting subpopulation (Florida) of loggerhead turtles produces predominately female hatchlings (Mrosovsky 1994; TEWG 2000), rookery beaches north of this region (Virginia to northern Florida) appear to be important sites for the production and recruitment of male turtles into the overall western North Atlantic population. Our data, although limited, indicate that even during the warmest nesting seasons, the ratio of male turtles produced from nests incubated on Georgia beaches is greater than that produced on rookery beaches south of our area. Moreover, in some seasons (herein represented by 2003 data) the sex ratio produced in Georgia is nearly 1:1. Such may also be the case for beaches north of our region, and, as a result, particular protection should be afforded to productive nesting beaches in this region to facilitate the necessary recruitment of male turtles into the overall western North Atlantic loggerhead population.

Our data indicate that male production is greatest in nests deposited earlier in the nesting season. Thus, nest monitoring programs in our region should take considerable steps to protect these nests in particular, including relocating nests in danger of tidal inundation to higher ground. Nest relocation practices are documented to have no adverse effects on the condition of hatchlings when compared with the condition of hatchlings from in situ nests (Tuttle and Rostal 2010), and this technique should be implemented to afford early season nests the opportunity to produce the greatest number of male turtles possible.

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